Irreversible Enzyme Inhibitors. CLVI.[1,2] Proteolytic Enzymes. XII.[3] Inhibitors of Guinea Pig Complement Derived by Quaternization of 3-Acylamidopyridines with α-Bromomethylbenzenesulfonyl Fluorides

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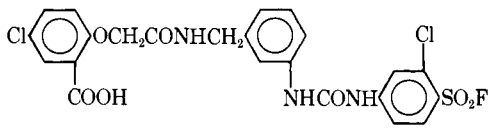
Received February 20, 1969

Twenty-eight quaternary salts derived from 3-acylamidopyridines or 3-(acylamidomethyl)pyridines by reaction with methyl or substituted-benzyl halides were investigated for inhibition of the lysis of sheep red blood cells by hemolysin and complement from the guinea pig. The most effective compounds were 3-(3,4-dichlorophenoxyacetamido)-N-(*p*-fluorosulfonylbenzyl)pyridinium bromide (**3**) and its *m*-SO_2F isomer (**4**) which showed about 50% inhibition at 0.5 m*M*.

The complement system is a complex mixture of serum proteases used for rejection of foreign cells by lysis and thus can also reject foreign organ or tissue transplants.[5] Since the complement system has both "tryptic" and "chymotryptic" properties,[5] it can be inhibited, as measured by complement–antibody-mediated lysis of sheep red blood cells (RBC), by some inhibitors of trypsin[6] or chymotrypsin.[3] Among the latter type is **1**, which is a good irreversible inhibitor of chymotrypsin,[7] and which at 0.25 m*M* gave 82% inhibition[3] of the complement–hemolysin–RBC system.[8,9] Since compounds of type **2** are also excellent irreversible inhibitors of chymotrypsin,[10] these quaternary salts have now been measured as inhibitors of the complement system; the results, along with results on some new related synthetics, constitute the subject of this paper.



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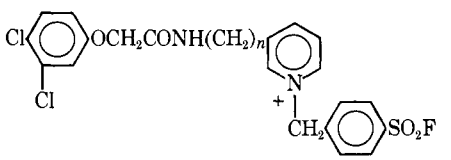

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Inhibition Results.—The data in Tables I and II are recorded as the effect of a given concentration of compound on lysis catalyzed by complement compared to a control with no compound. In some cases, acceleration of lysis is observed, which is expressed as a minus amount of inhibition. One cause of increased lysis of the RBC compared to the control was lysis caused by the compound in the absence of complement, expressed as a percentage of the total lysis possible, 0.7 OD unit. Another cause of acceleration was a direct effect on the complement system, as shown by a negative inhibition of complement and little or no lysis in the absence of complement.[6]

At a concentration of 0.5 m*M*, the sulfonyl fluoride (**3**) showed 45% inhibition of complement with no lysis in the absence of complement. However, at 1 m*M* **3** showed 45% lysis in the absence of complement and no inhibition in the presence of complement; these results with 1 m*M* **3** indicate that both compound-induced lysis and inhibition of complement-induced lysis are occurring. When the SO_2F moiety was moved to the *meta* position (**4**), compound lysis at 1 m*M* was considerably reduced; at 0.5 m*M*, **4** was somewhat more effective than **3** in inhibiting complement. Replacement of the CH_2 bridge to the pyridine N of **3** by CH_2CONH gave a compound (**5**) of reduced solubility that showed no inhibition of lysis at its maximum solubility of 0.12 m*M*.

When a methylene group was inserted between the carboxamide and pyridine moieties of **3** or **4**, the resultant **6** and **7** showed little lysis in the absence of complement, but at 0.5 m*M* were less effective inhibitors than **3** and **4**. Again, insertion of a CH_2CONH bridge to the benzenesulfonyl fluoride moiety gave a relatively insoluble compound (**8**) which showed no inhibition at its maximum solubility of 0.12 m*M*.

A study was then made to determine the effect of the tolylsulfonyl fluoride moiety of **3** and **4** on inhibition of complement. Removal of the SO_2F group resulted in **10** which had considerably reduced activity compared to **3** and **4**; that the benzyl group of **10** was necessary for its moderate activity was shown by comparison of the methyl analog (**9**) which showed no inhibition of complement. Attempts to study substituent effects on the benzyl binding (**11**–**15**) were hampered by lack of solubility; however, it was clear that (a) *p*-OCH_3 (**12**) or 3,4-Cl_2 (**15**) moieties gave an increase in compound-induced lysis in the absence of complement, (b) a *p*-NO_2 substituent (**13**) could not replace the *p*-SO_2F group of **3** for maintenance of inhibition, and (c) the effect of a *p*-CH_3 (**11**) or *m*-NO_2 group (**14**) could not be ascertained with certainty due to lack of solubility, but there was no appreciable increase in effectiveness.

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series see B. R. Baker and E. E. Janson, *J. Med. Chem.*, **12**, 672 (1969).

(3) For the previous paper on complement see B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 415 (1969), paper CLIII of this series.

(4) NDEA predoctoral fellow.

(5) (a) Ciba Foundation Symposium, Complement, G. E. W. Wolstenholme and J. Knight, Ed., Little, Brown and Co., Boston, Mass., 1965; (b) H. J. Müller-Eberhard, *Advan. Immunol.*, **8**, 1 (1968); (c) P. H. Schur and K. F. Austen, *Ann. Rev. Med.*, **19**, 1 (1968).

(6) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **12**, 408 (1969), paper CLII of this series.

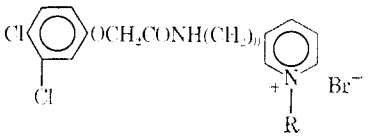
(7) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 118 (1969), paper CXLV of this series.

(8) E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," 2nd ed, Charles C Thomas, Springfield, Ill., 1967, pp 149–153.

(9) For details of the assay used in this laboratory, see ref 6.

(10) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 221 (1969), paper CL of this series.

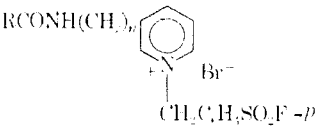
Table I
Inhibition[a,b] of Guinea Pig Complement by



No.[e]	n	R	Concn, mM	% inhibn[c]	% lysis[d]
3	0	$CH_2C_6H_4SO_2F$-*p*	1	−5	45
			0.5	45	4
			0.25	12	
4	0	$CH_2C_6H_4SO_2F$-*m*	1	61	17
			0.5	58	7
5	0	$CH_2CONHC_6H_4SO_2F$-*p*	0.125[f]	0	7
			0.062	0	
6	1	$CH_2C_6H_4SO_2F$-*p*	1	56	10
			0.5	19	
7	1	$CH_2C_6H_4SO_2F$-*m*	1	63	7
			0.5	33	
8	1	$CH_2CONHC_6H_4SO_2F$-*p*	0.125[f]	−5	15
			0.062	0	
			0.031	0	
9	0	CH_3	1	−14[g]	12
			0.5	−20	
			0.25	−10	
10	0	$CH_2C_6H_5$	1[f]	27	11
			0.5	20	
11	0	$CH_2C_6H_4CH_3$-*p*	0.25[f]	20	2
12	0	$CH_2C_6H_4OCH_3$-*p*	1		100
			0.5	21	11
13	0	$CH_2C_6H_4NO_2$-*p*	0.5[f]	14	6
			0.25	9	
14	0	$CH_2C_6H_4NO_2$-*m*[h]	0.25[f]	6	0
			0.125	0	
15	0	$CH_2C_6H_3Cl_2$-3,4[h]	0.25[f]	−70	73
			0.125	0	
			0.062	0	
16	1	CH_3[i]	1	6	3
			0.5	−6	
17	1	$CH_2C_6H_5$	3	16	
			1	0	5
			0.5	5	
18	1	$CH_2C_6H_4OCH_3$-*p*	1	5	
			0.5	−7	6
19	1	$CH_2C_6H_4NO_2$-*p*	1	0	15
			0.5	0	

[a] The technical assistance of Sharon Lafler with these assays is acknowledged. [b] See ref 6 for assay of inhibition of sheep red blood cell lysis by hemolysin and guinea pig complement. The compounds were dissolved in either MeOEtOH or 4:1 MeOEtOH–H_2O for assay. [c] A minus number indicates more lysis than the complement control without compound. [d] Lysis in the absence of complement corrected for 2–5% lysis in the absence of compound. [e] See ref 10 for synthesis. [f] Maximum solubility. [g] Iodide salt. These results may not be reliable since red blood cells were discolored by the compound; 1 m*M* NaI gave no lysis or discoloration. [h] Chloride salt. [i] Tosylate salt.

Table II
Inhibition[a,b] of Guinea Pig Complement by



No.	n	R	Concn, mM	% inhibn[c]	% lysis[d]
20	0	$C_6H_5OCH_2$	2	49	8
			1	27	6
			0.5	15	
21	0	$C_6H_5CH_2$	2	51	10
			1	34	4
			0.5	16	
22	0	C_6H_5	2	53	6
			1	28	7
			0.5	14	
23[e]	0	CH_3	3	67	0
			1	21	
			0.5	5	
24	0	$2\text{-}ClC_6H_4OCH_2$	1[f]	38	0
			0.5	14	
25	0	$3\text{-}ClC_6H_4OCH_2$	1	58	8
			0.5	25	
26	0	$4\text{-}ClC_6H_4OCH_2$	1	64	0
			0.5	19	
27	0	$2,4\text{-}Cl_2C_6H_3OCH_2$	1	37	9
			0.5	51	0
			0.25	0	
28	0	$2,3\text{-}Cl_2C_6H_3OCH_2$	1	−87	100
			0.5	52	0
			0.25	21	
29	0	$\beta\text{-}C_{10}H_7OCH_2$	0.025[f]	0	0
			0.0125	0	
30	0	L-$C_6H_5CH_2CHNHSO_2$-$C_6H_4CH_3$-*p*	0.25[f]	14	3
			0.125	7	1
31	1	L-$C_6H_5CH_2CHNHSO_2$-$C_6H_4CH_3$-*p*	1	37	9
			0.5	21	4

[a–d] See corresponding footnotes in Table I. [e] See ref 10 for synthesis. [f] Maximum solubility.

The substituent effects in the pyridylmethylene series (**6**, **7**) were more clear cut since little lysis in the absence of complement is seen with **6**, **7**, or **16**–**19** and solubility is greater. The lack of inhibition at 1 m*M* when the *p*-SO_2F moiety of **6** is replaced by H (**17**), OCH_3 (**18**), or NO_2 (**19**) showed the unique effect of the SO_2F group which cannot necessarily be attributed to the ability of the SO_2F group to form a covalent bond with one or more of the components of the complement system by the active-site-directed mechanism.[10,11] Further extensive studies of the type previously suggested[3] for SO_2F-type inhibitors of complement would be necessary to establish this important point.

The effect of the 3,4-dichlorophenoxyacetyl moiety of **3** on inhibition of complement was then studied (Table II). Removal of the two Cl's gave **20** which required a fourfold increase in concentration to give the same inhibition as **3** showed at 0.5 m*M*. Replacement of the phenoxyacetyl moiety of **20** by phenylacetyl (**21**), benzoyl (**22**), or acetyl (**23**) gave little change in activity.

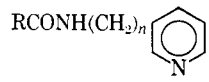
Since the two Cl's of **3** gave enhanced activity over **20**, other chloro substitution was investigated. The 3-Cl (**25**) and 4-Cl (**26**) derivatives were about half as effective as the 3,4-Cl_2 derivative (**3**), but the 2-Cl (**24**) was even less effective. The 2,4-Cl_2 (**27**) and 2,3-Cl_2 (**28**) derivatives were as effective as **3**. The β-naphthoxy derivative (**29**) was too insoluble to determine an effective inhibition level.

Replacement of the 3,4-dichlorophenoxyacetyl moiety of **6** by the more natural N-tosyl-L-phenylalanyl moiety gave **31** which was somewhat less effective than **6** as an inhibitor of complement; however, **31** was more

(11) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.

TABLE III

PHYSICAL PROPERTIES OF

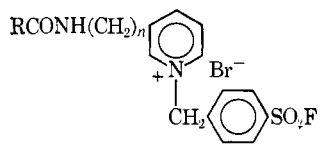


No.	n	R	Method[a]	% yield[b]	Mp, °C	Formula[c]
36	0	$C_6H_5OCH_2$	A	66	106–108	$C_{13}H_{12}N_2O_2$
37	0	2-$ClC_6H_4OCH_2$	A	45	111–113	$C_{13}H_{11}ClN_2O_2$
38	0	3-$ClC_6H_4OCH_2$	A	65	130–132	$C_{13}H_{11}ClN_2O_2$
39	0	4-$ClC_6H_4OCH_2$	A	97	128–130	$C_{13}H_{11}ClN_2O_2$
40	0	2,4-$Cl_2C_6H_3OCH_2$	A	68	135–137	$C_{13}H_{10}Cl_2N_2O_2$
41	0	2,3-$Cl_2C_6H_3OCH_2$	A	49	143–147	$C_{13}H_{10}Cl_2N_2O_2$
42[d]	0	2,4,6-$Cl_3C_6H_2OCH_2$	A	44	127–132	$C_{13}H_9Cl_3N_2O_2$
43	0	β-$C_{10}H_7OCH_2$	A	27	143–146	$C_{17}H_{14}N_2O_2$
44	0	L-$C_6H_5CH_2CHNHSO_2C_6H_4CH_3$-*p* |	B[e]	21[f,g]	245–248	$C_{21}H_{21}N_3O_3S\cdot HCl$
45	1	L-$C_6H_5CH_2CHNHSO_2C_6H_4CH_3$-*p* |	A[e]	66	160–165	$C_{22}H_{23}N_3O_3S$

[a] A: acylation of **32** with appropriate acid chloride in $CHCl_3$ at 0° containing Et_3N as previously described;[10] B: a 2:1 excess of amine used as acid acceptor in place of Et_3N. [b] Yield of pure product after recrystallization from toluene unless otherwise indicated. [c] Analyzed for C, H, N. [d] Reaction with **34** gave an oil that could not be purified. [e] See E. A. Popenoe and V. du Vigneaud, *J. Am. Chem. Soc.*, **76**, 6202 (1954), for preparation of intermediate acid chloride. The HCl salt of **44** was crystallized from 5% HCl. [f] Recrystallized from H_2O, then EtOH. [g] The free base, mp 152–154°, was prepared in 100% yield by adjusting a solution in Me_2CO–H_2O to pH 8.

TABLE IV

PHYSICAL PROPERTIES OF



No.	n	R	Yield,[a] %	Mp, °C dec	Formula	Analyses
20	0	$C_6H_5OCH_2$	71[b]	203–205	$C_{20}H_{18}BrFN_2O_4S$	C, H, F
21	0	$C_6H_5CH_2$	70[c,d]	189–193	$C_{20}H_{18}BrFN_2O_3S$	C, H, F
22	0	C_6H_5	72[c,e]	156–158	$C_{19}H_{16}BrFN_2O_3S\cdot CH_3COCH_3$[f]	C, H, F
24	0	2-$ClC_6H_4OCH_2$	54[b]	217–220	$C_{20}H_{17}BrClFN_2O_4S$	C, H, F
25	0	3-$ClC_6H_4OCH_2$	71[b]	191–193	$C_{20}H_{17}BrClFN_2O_4S$	C, H, F
26	0	4-$ClC_6H_4OCH_2$	81[g]	231–233	$C_{20}H_{17}BrClFN_2O_4S$	C, H, F
27	0	2,4-$Cl_2C_6H_3OCH_2$	62[b,h]	191–193	$C_{20}H_{16}BrCl_2FN_2O_4S$	C, H, F
28	0	2,3-$Cl_2C_6H_3OCH_2$	51[b]	163–166	$C_{20}H_{16}BrCl_2FN_2O_4S$	C, H, F
29	0	β-$C_{10}H_7OCH_2$	56[i]	229–230	$C_{24}H_{20}BrFN_2O_4S$	C, H, F
30	0	L-$C_6H_5CH_2CHNHSO_2C_6H_4CH_3$-*p* |	22[j,c]	150–155	$C_{28}H_{27}BrFN_3O_5S\cdot 0.5H_2O$	C, H, N
31	1	L-$C_6H_5CH_2CHNHSO_2C_6H_4CH_3$-*p* |	18[c]	198–200	$C_{29}H_{29}BrFN_3O_5S_2$	C, H, F

[a] Compounds synthesized by condensation of the appropriate pyridine with **34** in warm Me_2CO as previously described;[10] yields are of pure product. [b] Recrystallized from EtOH. [c] Recrystallized from Me_2CO. [d] The starting pyridine was prepared by method A; an alternate method has been described by A. Buzas, F. Conac, C. Egnell, and P. Freon, *Compt. Rend.*, **C262,** 658 (1966). [e] The starting pyridine was made by method A; an alternate method has been described by H. Binz and C. Rath, *Ann.*, **486**, 95 (1931). [f] Me_2CO verified by ir and nmr. [g] Recrystallized from H_2O–EtOH. [h] Recrystallized from EtOH–Me_2CO. [i] Recrystallized from MeOEtOH–Me_2CO. [j] The compound was precipitated from the reaction mixture with petroleum ether (bp 30–60°), then recrystallized from H_2O.

effective than N-benzoyl-L-tyrosine ethyl ester.[3] The lower homolog (**30**) was too insoluble to determine an effective inhibition level.

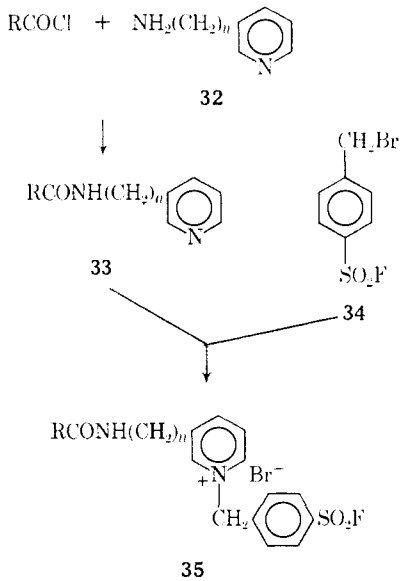
The two L-phenylalanine derivatives (**30**, **31**) were also investigated as irreversible inhibitors of α-chymotrypsin. Both had $K_i \sim 20\ \mu M$ and at a K_i concentration gave complete inactivation of α-chymotrypsin ($\sim 1\ \mu M$) in 2–3 min at 37°, about the same as observed

with **3** and **6**.[10] Thus, the 3,4-dichlorophenoxyacetyl group is equivalent to the N-tosyl-L-phenylalanyl group for reversible and irreversible inhibition of α-chymotrypsin, but the latter seems less effective for inhibition of complement.

The two most effective compounds in Tables I and II were **3** and **4**; however, these were less effective than **1**.[3] Many questions are raised by these initial studies that should be answered. (1) Can substituent effects on the C_6H_5 moiety of **21** and **22**, as well as additional substituent studies related to **24–29**, lead to enhanced activity? (2) What is the optimum number of methylenes between the amide and pyridyl moieties of **3** and **6**? With two or more methylenes the 2- and 4-pyridyl isomers can also be synthesized for evaluation, since these isomers of **3** and **6** cannot be synthesized due to instability.[10] (3) Could other bridges between the pyridinium N and the phenylsulfonyl fluoride moiety lead to enhanced activity? (4) Would a study of substituent effects on the phenylsulfonyl fluoride moiety be rewarding? (5) Would substitution on the amide N be beneficial?

Studies to answer some of these questions are currently proceeding in this laboratory.

Chemistry.—The synthesis of compounds in Table I have been described previously;[10] those in Table II were made by the same general route *via* **33** by quaternization with **34**.[12]



The physical properties of **36–45** and **20–22** and **24–31** are given in Tables III and IV, respectively.[13]

(12) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 666 (1968), paper CXXVII of this series.

(13) Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had the appropriate ir spectrum, moved as a single spot on tlc on Brinkmann silica gel GF (Table III) or polyamide MN (Table IV), and gave combustion values for C, H, and N or F within 0.4% of theory.

Irreversible Enzyme Inhibitors. CLVII.[1,2] Effect of Bridge Modification on the Selective Irreversible Inhibition of Dihydrofolic Reductase from L1210 Mouse Leukemia and Liver by 2,4-Diamino-5-(3,4-dichlorophenyl)-6-[*p*-(*m*-fluorosulfonylbenzamidomethyl)phenoxymethyl]pyrimidine. I

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Received April 1, 1969

The title compound (**1**) is an active-site-directed irreversible inhibitor of the dihydrofolic reductase from L1210 mouse leukemia that shows specificity by not inactivating this enzyme from normal mouse liver; however, **1** had $K_i = 0.06\ \mu M$ which was considered too large for *in vivo* effectiveness. Twenty-eight related compounds with and without substituents on one of the two 6-phenyl moieties have now been investigated; six compounds had $K_i < 0.01\ \mu M$ and showed good irreversible inhibition of the L1210 enzyme, but specificity was decreased or lost. The ability of these 29 compounds to inhibit L1210 cell culture (ED_{50}) was investigated. When ED_{50}/I_{50} was used as an approximate estimation of transport through the cell wall, the best compound was 2,4-diamino-5-(3,4-dichlorophenyl)-6-[4-(*m*-fluorosulfonylphenylureido)-3-methylphenoxymethyl]pyrimidine (**16**) with $ED_{50} = 0.05\ \mu M$ and $ED_{50}/I_{50} = 2$. However, **16** was still two to three magnitudes less effective than 2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine with $ED_{50} = 2 \times 10^{-5}\ \mu M$ and $ED_{50}/I_{50} = 0.002$. From the differences in ED_{50}/I_{50} ratios, substitution of a 2-MeO or 3-Me group in the phenoxy moiety enhanced transport in half the cases; in most cases the compounds with a urea bridge showed better transport than the corresponding compounds with an amide bridge.

Several types of active-site-directed irreversible inhibitors,[4] bearing a terminal SO_2F moiety, have been found for dihydrofolic reductase that can inactivate the enzyme from L1210 mouse leukemia with no inactivation of this enzyme from normal mouse liver.[5-7] One of these isozyme-specific irreversible inhibitors was **1**.[7] However, **1** failed to meet the three criteria[5]

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series see B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 677 (1969).

(3) N. M. J. V. wishes to thank the Council for Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

(4) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.

(5) (a) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 489 (1968), paper CXIX of this series; (b) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 108 (1969), paper CXLIII of this series; (c) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 224 (1969), paper CLI of this series.

(6) B. R. Baker and G. J. Lourens, *ibid.*, **12**, 95 (1969), paper CXL of this series.

(7) B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968), paper CXX of this series.

(8) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXXIII of this series.